RESEARCH ARTICLE

Mouse Plantar Flexor Muscle Size and Strength After Inactivity and Training

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Introduction: Losses in muscle mass and strength may affect an astronaut's safety; therefore, it is of utmost importance to optimize countermeasures to minimize atrophy and strength loss during spaceflight. The main purpose of this study was to determine if high force eccentric or isometric contractions performed by the plantar flexor group during hind limb suspension would preserve muscle mass and strength. ***Methods:*** Plantar flexor muscles of mice were trained with either eccentric or isometric contractions every other day during a 10-d hind limb suspension period. Pre- and post-suspension stimulation frequency- and angular velocity-dependent measurements of torque of the plantar flexors, soleus twitch (P_t) and tetanic (P_o) force, bodyweight, and muscle wet weight measurements were made. ***Results:*** The 19 and 26% losses in gastrocnemius and soleus muscle wet weights, respectively, were not attenuated with eccentric or isometric contractions. Neither eccentric nor isometric contractions attenuated the soleus muscle's 30% isometric force loss after hind limb suspension. Despite losses in muscle mass, there was no decrease in the force produced by the plantar flexor muscle group after hind limb suspension. ***Discussion:*** Hind limb suspension decreased both gastrocnemius and soleus mass, and in vitro soleus force production. However, in vivo force production of the plantar flexor muscle group did not decrease, which may be explained by a shift in the isometric torque: ankle angle relationship. The use of eccentric or isometric contractions as a countermeasure to offset muscle mass and strength requires further investigation as neither was capable of maintaining soleus muscle force production, or gastrocnemius and soleus muscle mass during hind limb suspension.

Keywords: eccentric contraction, microgravity, exercise.

SPACEFLIGHT CAUSES atrophy and strength loss in antigravity skeletal muscles. The loss of size and function could compromise performance in space or jeopardize astronaut safety in an emergency upon returning to gravity. Resistance exercise has been proposed to be an efficient means to decrease muscle atrophy during spaceflight (4). For example, Kirby et al. (15) demonstrated that 24 eccentric contractions performed every other day during 10 d of hind limb suspension prevented 74 and 44% of the soleus muscle (SOL) wet weight and protein content losses, respectively, a countermeasure that took less than 1 min/d. More recently, Adams et al. (2) used a combination of isometric, concentric, and eccentric contractions to minimize atrophy of the medial gastrocnemius muscle (GAST) during 5 d of hind limb suspension. This finding is important because in rodents, even though the SOL atrophies to a greater relative extent than the GAST during unloading (18,24), the absolute amount of wet weight and protein content lost in the GAST is much greater because the GAST is at least 10-fold larger than the SOL (3,7). Therefore, preventing atrophy of the faster muscles could potentially be more important in terms of enhancing performance during an emergency. Interestingly, although eccentric contractions preserved muscle mass in the rat SOL (15) and a combination of isometric, concentric, and eccentric contractions preserved muscle mass in the rat medial gastrocnemius (2), the use of isometric resistance exercise alone, which has been previously shown to cause hypertrophy (1), was unable to maintain muscle mass during 5 d of hind limb suspension.

Although these studies support the idea that resistance training may be beneficial for minimizing skeletal muscle atrophy, it is difficult to ascertain the physiological importance since no measurements of force-producing capacity were made. Also, it is unclear as to whether it is the very high load placed on the muscle during the different contraction types, especially during eccentric contractions, that prevents the atrophy, or if it is something inherent to the kinematic properties of the differing contraction types (e.g., lengthening vs. shortening) that make them beneficial. The purpose of this study was to test the following hypotheses: 1) in vivo force loss of the plantar flexors during hind limb suspension is proportional to muscle atrophy; 2) high force eccentric or isometric contractions performed periodically by the plantar flexor group in mice during hind limb suspension would decrease muscle atrophy and force loss; and 3) the extent of attenuation of atrophy and force loss is proportional to the forces produced by the muscles during the countermeasures.

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METHODS

Animals

There were 49 female ICR mice (8-10 wk, Harlan) that were assigned to 1 of 6 groups: 1) normal cage activity (CON-NON); 2) hind limb suspended and no countermeasure (CON-SUS); 3) hind limb suspended and isometrically trained at 100% of maximal isometric torque (ISO-100); 4) hind limb suspended and isometrically trained at 80% of maximal isometric torque (ISO-80); 5) hind limb suspended and eccentrically trained at 140% of maximal isometric torque (ECC-140); and 6) hind limb suspended and eccentrically trained at 100% of maximal isometric torque (ECC-100). The mice were housed 4-5 per 24 $\times$ 46 cm polycarbonate cage prior to hind limb suspension and individually during the hind limb suspension or ambulatory control treatment. During all conditions, the mice had access to food and water ad libitum and were housed in a 20-23°C room with a 12-h dark-light period. All animal care and use procedures were approved by Texas A&M University Laboratory Animal Care Committee.

Experimental Design

All mice were implanted with a stimulating nerve cuff on the left tibial nerve, which innervates the plantar flexor muscles (i.e., GAST, plantaris, and SOL), and were allowed to recover for 4-5 wk. After the recovery from implantation surgery, all mice were anesthetized and performed a prophylactic conditioning bout of 30 isometric and 30 eccentric contractions of the plantar flexor muscles using a computer controlled servomotor 1 wk prior to initiation of hind limb suspension. Pilot work determined this was adequate conditioning to prevent contraction-induced damage associated with eccentric contractions during the subsequent training bouts. Immediately before the hind limb suspension, stimulation frequency- and angular velocity-dependent measurements of torque produced by the plantar flexor muscles at the ankle and the respective exercise countermeasure were performed. All mice were anesthetized every other day during the 10-d period of hind limb suspension or control cage activity. On these days, the mice were secured to an in vivo torque measurement apparatus and had the proximal leads of their nerve cuffs externalized in the dorsal cervical region. The countermeasure groups then performed the respective exercise countermeasure protocols. At the end of the 10-d suspension, in vivo stimulation frequency- and angular velocity-dependent torques were measured, as were twitch (P_t) and tetanic (P_o) isometric forces in the in vitro SOL preparation. Both the GAST and SOL muscles were weighed and frozen.

Nerve Cuff Implantation

A modification of the technique described by Warren et al. (26) was used to construct and implant a nerve cuff on the left tibial nerve. Briefly, the nerve cuff was constructed out of Teflon-coated, multi-stranded 90% Pt-10% Ir wire (Medwire-Sigmund Cohn, Mt. Vernon, NY) under a dissecting microscope. Mice were anesthetized with pentobarbital sodium (100 mg $\cdot$ kg^{-1} ip), and the left leg and dorsal cervical area were shaved and aseptically prepared. A small incision was made through the skin and biceps femoris muscle over the area where the sciatic nerve trifurcates. The tibial nerve was carefully separated from the other nerves and fascia with a fine tip glass rod and placed inside the nerve cuff loops. The loops were closed and the proximal leads of the nerve cuff were externalized in the dorsal cervical region. The nerve cuff was secured to the fascia of the biceps femoris muscles, and the muscle and skin incisions were closed using 6-0 silk sutures. A 9-mm wound clip was used to close the dorsal cervical skin incision. The mouse was then returned to its cage to recover.

In Vivo Torque Measurements and Exercise Training

Torque measurements and training of the plantar flexor muscles was done on a miniature isokinetic dynamometer (computer-controlled 300B servomotor, Cambridge Technology, Huntington Beach, CA; S48 stimulator, Grass Instruments, West Warwick, RI; Testpoint version 4.0B software, Capital Equipment, Billerica, MA; and a KPCI-3108 A/D board, Keithley Instruments, Cleveland, OH) as described previously (16). First, the mouse was anesthetized with intraperitoneal injections of fentanyl (0.33 mg $\cdot$ kg^{-1}), droperidol (16.7 mg $\cdot$ kg^{-1}), and diazepam (5 mg $\cdot$ kg^{-1}), and the skin over the dorsal cervical region was shaved and aseptically prepared. The proximal end of the nerve cuff was externalized and the mouse was secured to the torque measurement apparatus with the knee and ankle joint angles set at 90° angles. The stimulation voltage was adjusted using 200-ms trains of 0.1-ms pulses at 300 Hz to yield the maximal isometric torque of the plantar flexor muscles at the ankle.

The pre- and post-suspension torque measurements consisted of a sequence of 11 200-ms isometric contractions with 0.1-ms pulses at the following stimulation frequencies of 10, 20, 40, 60, 80, 100, 125, 150, 200, 250, and 300 Hz, and a sequence of 7 concentric contractions and 1 isometric contraction at the following angular velocities of 1200, 1000, 800, 600, 400, 200, 100, and 0° $\cdot$ s^{-1}. All isometric contractions were performed at an ankle joint angle of 90°. All concentric contractions were performed over a 40° arc (from a joint angle of 70° to 110°) at a stimulation frequency of 150 Hz. The muscles were only stimulated for the duration necessary to complete the movement. There was a 45-s rest interval between contractions.

Torque was also measured at different ankle angles to test for hind limb suspension-induced changes in the isometric torque:ankle angle relationship. Seven mice were implanted with a nerve cuff and hind limb-suspended for 10 d. Isometric torque (200-ms train of 0.1-ms pulses at 300 Hz) was measured both before and after suspension at nine angles between 20° of plantarflexion and 20° of dorsiflexion.

The in vivo training bouts, which consisted of 30 isometric or eccentric contractions with 12-s rest intervals between contractions, were performed 5 times by the mice in the countermeasure groups, i.e., immediately before the initiation of hind limb suspension and every other day during the suspension. An every other day training protocol was chosen because of the success Kirby et al. (15) had in attenuating atrophy during hind limb suspension in rats with the every other day schedule; also, we felt that the stress of daily anesthesia could compromise the health of the mice. The amount of torque produced by the plantar flexor muscles was adjusted to the prescribed amount by varying the stimulation frequency for each training session. See **Fig. 1** for the average stimulation frequencies used and the peak torque values for the training groups during the five sessions. For the eccentric contractions, the foot was passively moved from the neutral position (i.e., 90° between the plantar surface and the tibial bone long axis) to a 20° plantar-flexed position. The muscles were then stimulated while the servomotor moved the foot at $200° \cdot s^{-1}$ over a 40° range of motion (i.e., from 20° of plantarflexion to 20° of dorsiflexion).

Hind Limb Suspension

While still under anesthesia from the initial in vivo torque measurements and training (if applicable), the mice were hind limb suspended using a procedure described previously (25). This procedure allowed the individually housed mice to ambulate, groom, and feed themselves using their forelegs. For the bodyweight measurements and exercise training, the mice were anesthetized while suspended and were re-suspended before the anesthesia wore off. Thus, the hind limbs were never loaded by gravity during the experimental period. Because most of the atrophy to the SOL occurs during the first week (13,14) and suspension periods that have ranged from 7 to 14 d have previously been used to test countermeasure effectiveness in mice and rats (7,11,15), 10 days of suspension was chosen. There were no differences in bodyweights among groups on the day of hind limb suspension; the mean (± SEM) body weight at that time was 28.9 ± 0.3 g.

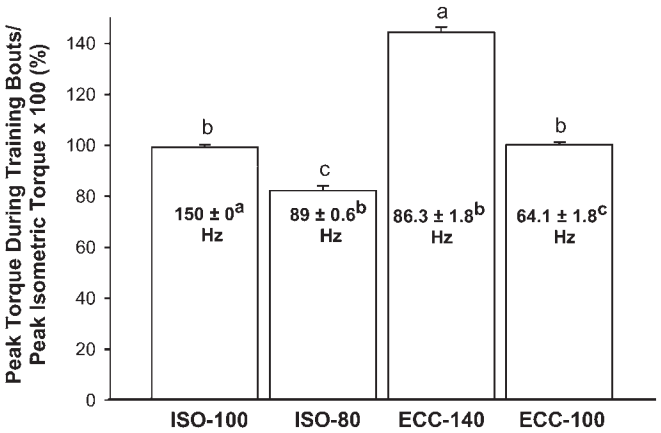

Fig. 1. Mean (± SEM) peak torques during the five training bouts as a percentage of the peak isometric torque at 300 Hz immediately prior to the training bouts. During each bout the stimulation frequency was varied to achieve a percentage of the peak isometric torque that was obtained at 300 Hz immediately prior to the training bouts. The mean stimulation frequencies (± SEM) used to obtain the torques are contained within the bar chart. An "a" indicates the group is significantly different from groups labeled with a "b" or "c." A "b" indicates the group is significantly different from groups with an "a" or "c," and a "c" indicates the group is significantly different from groups labeled with an "a" or "b"; $P < 0.05$.

SOL Mechanics

The SOL P_t and P_o forces were determined in vitro with a preparation similar to that previously described (13,25). The mouse was anesthetized with pentobarbital sodium ($100 \text{ mg} \cdot \text{kg}^{-1}$ ip), and the GAST and plantaris were carefully removed to expose the SOL. The SOL was dissected free and a silk suture connected to the distal tendon was secured to the base of the glass incubation chamber, which contained oxygenated Krebs-Ringer bicarbonate buffer maintained at 35°C. The proximal tendon was attached by silk suture to the lever arm of a position-feedback computer controlled-servomotor (Cambridge Technology model 300B). Muscle length was set by adjusting the resting force to 4.5 mN and measured using a sighting scope. This passive force corresponds to the resting force of the SOL at anatomical L_o (25). There were no differences in the mean resting length among groups (14.5 ± 0.1 mm). Two P_t and two P_o contractions (400-ms trains with 0.2-ms pulses at 150 Hz) were performed with 30 s between contractions except for between the two P_o contractions when the interval was 2 min. After these measurements were performed, the passive force was increased and decreased (± 20%) by lengthening and shortening the muscle; an increase in P_t was not observed with an increase in resting force. Therefore, the length used was deemed to be the optimal length. The GAST and SOL were weighed, frozen in liquid nitrogen, and stored at −80°C. Specific muscle force was calculated by normalizing P_o to physiological cross-sectional area (5).

Statistics

Bodyweight was analyzed with a group (CON-NON, CON-SUS, ISO-100, ISO-80, ECC-140, ECC-100) by day (0, 2, 4, 6, 8, 10) ANOVA with repeated measures on the day factor. The absolute and normalized isometric torques as a function of stimulation frequency were analyzed with day (pre- and post-suspension) by group by frequency (10, 20, 40, 60, 80, 100, 125, 150, 200, 250, 300 Hz) ANOVAs with repeated measures on day and frequency. The absolute and normalized torques as a function of angular velocity were analyzed with day by group by velocity (1200, 1000, 800, 600, 400, 200, 100, $0° \cdot s^{-1}$) ANOVAs with repeated measures on day and velocity. One-way ANOVAs were used to analyze the following data: absolute and normalized muscle wet weights, SOL lengths, GAST protein contents, absolute and normalized SOL P_ts and P_os, SOL specific forces, and the peak torques, average torques, and stimulation frequencies during training bouts. Student-Newman-Keuls post hoc

tests were performed when significant main effects or interactions were found. An α level of 0.05 was used for all analyses. All values are presented as mean ± SEM.

RESULTS

The post-suspension bodyweights of the hind limb suspended groups (except ECC-100) were ~6% less than those of the CON-NON group (**Table I**). Since the ECC-100 group lost more bodyweight (12%) than any of the other hind limb suspended groups, the analyses of forces, torques, and muscle wet weights were also done after normalizing to bodyweight. GAST and SOL absolute wet weights of all hind limb suspended mice decreased 13 and 20%, respectively, than those in the CON-NON group, and there were no differences among suspended groups. When normalized by bodyweight there were no differences in GAST wet weights among groups. The SOL normalized wet weights were 17–25% lower in all suspended groups, except for the ISO-100 group. The GAST total protein content of the CON-SUS group was 21% lower than that of the CON-NON group (data not shown).

SOL P_o, both absolute and normalized to bodyweight values, were 26–36% and 21–33% lower, respectively, in all suspended groups than those in the CON-NON group, and there were no differences among the suspended groups. Also, there were no differences in SOL P_t, SOL P_t/P_o, and SOL specific force among the groups. This indicates that none of the countermeasures were effective in preventing the force loss in the SOL muscle during the hind limb suspension.

The mean peak torque production as a percentage of the peak isometric torque at 300 Hz was ~40% greater in the ECC-140 group than in the ISO-100 and ECC-100 groups, and the mean peak torque production of the ISO-80 group was ~80% of the mean peak torque production of the ISO-100 group (Fig. 1). Mean stimulation frequencies to obtain the different peak torques were different between all groups except for ISO-80 and ECC-140 (Fig. 1). These data indicate that by changing stimulation frequency torque production was controlled during different muscle actions during the training bouts.

There was a decrease in the absolute torque in ECC-100 as compared to CON-NON between 125 and 300 Hz, but there were no differences among groups in the frequency-dependent torque produced by the plantar flexors when normalized to bodyweight (**Fig. 2**). The absolute peak torques produced during concentric contractions after suspension were only reduced in the ECC-100 group when compared to the pre-suspension values, and the differences were only at the lower velocities (100 and 200° · s^{-1}). All suspension groups had lower absolute torques during the 100° · s^{-1} contractions when compared to the CON-NON group; the CON-SUS torque was 10% lower than the CON-NON torque at 100° · s^{-1} (**Fig. 3**). There was a significant effect of hind limb suspension on the angle:isometric torque relationship in the more dorsiflexed positions of animals in the CON-SUS group (**Fig. 4**). There was a significant suspension:angle interaction but the post-suspension isometric torque was only significantly affected (decreased by 19%) at the 20° dorsiflexed position (i.e., ankle angle of 70°).

TABLE I. BODYWEIGHT, MUSCLE WEIGHT, SOL LENGTH, SOL CONTRACTILE PROPERTIES, AND TRAINING BOUT STIMULATION FREQUENCY AND TORQUE PRODUCTION.

	CON-NON (N = 9)	CON-SUS (N = 9)	ISO-100 (N = 8)	ISO-80 (N = 7)	ECC-140 (N = 8)	ECC-100 (N = 8)
Pre bodyweight (g)	28.7 ± 0.5	29.2 ± 0.9	28.9 ± 0.7	29.0 ± 1.2	29.2 ± 0.8	28.6 ± 0.5
Post bodyweight (g)	28.1 ± 0.4^{a}	26.5 ± 0.7^{b*}	25.9 ± 0.9^{b*}	26.4 ± 1.2^{b*}	26.6 ± 0.6^{b*}	24.7 ± 0.3^{c*}
GAST wet weight (mg)	149.0 ± 6.7^{a}	119.6 ± 5.8^{b}	125.2 ± 7.4^{b}	119.0 ± 4.0^{b}	129.5 ± 4.8^{b}	128.9 ± 3.7^{b}
Norm. GAST wet weight (mg · g^{-1})	5.3 ± 0.2	4.5 ± 0.2	4.8 ± 0.2	4.6 ± 0.2	4.9 ± 0.2	5.2 ± 0.1
SOL wet weight (mg)	6.8 ± 0.3^{a}	5.0 ± 0.3^{b}	5.6 ± 0.3^{b}	4.6 ± 0.1^{b}	5.0 ± 0.2^{b}	4.9 ± 0.4^{b}
Norm. SOL weight (mg · g^{-1})	0.24 ± 0.01^{a}	0.19 ± 0.01^{b}	0.22 ± 0.01^{a,b}	0.18 ± 0.01^{b}	0.19 ± 0.01^{b}	0.20 ± 0.02^{b}
SOL P_t (mN)	23.9 ± 2.2	21.0 ± 1.4	23.5 ± 2.1	20.7 ± 2.4	22.0 ± 1.4	22.0 ± 1.4
SOL P_0 (mN)	161.6 ± 7.8^{a}	113.5 ± 10.2^{b}	119.1 ± 11.1^{b}	103.0 ± 8.3^{b}	110.1 ± 3.8^{b}	114.4 ± 8.1^{b}
Norm. SOL P_0 (mN · g^{-1})	5.8 ± 0.3^{a}	4.3 ± 0.3^{b}	4.6 ± 0.4^{b}	3.9 ± 0.3^{b}	4.1 ± 0.1^{b}	4.6 ± 0.3^{b}
SOL specific P_0 (N · cm^{-2})	26.9 ± 1.3	25.1 ± 0.8	23.0 ± 1.5	23.7 ± 1.8	24.6 ± 1.3	25.9 ± 1.2
P_t/P_0	0.15 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.01	0.20 ± 0.01
SOL length (mm)	14.8 ± 0.2	14.8 ± 0.2	14.5 ± 0.2	14.0 ± 0.4	14.6 ± 0.2	14.5 ± 0.2

Values are means ± SEM. CON-NON: normal cage activity; CON-SUS: hind limb suspended with no countermeasure; ISO-100: hind limb suspended and isometrically trained at 100% of maximal torque; ISO-80: hind limb suspended and isometrically trained at 80% of maximal isometric torque; ECC-140: hind limb suspended and eccentrically trained at 140% of maximal isometric torque; ECC-100: hind limb suspended and eccentrically trained at 100% of maximal isometric torque; GAST: medial gastrocnemius muscle; SOL: soleus muscle; P_t: twitch isometric force; P_o: tetanic isometric force.

The data in the table are listed in descending order for the following: pre-suspension bodyweight, post-suspension bodyweight, GAST wet weight, GAST wet weight normalized to bodyweight, SOL wet weight, SOL wet weight normalized to bodyweight, SOL twitch force, SOL tetanic force, SOL tetanic force normalized to bodyweight, SOL tetanic force normalized to physiological cross-sectional area, SOL twitch-tetanus ratio, and SOL resting length. An "a" indicates the group is significantly different from groups labeled with a "b" or "c." A "b" indicates the group is significantly different from groups with an "a" or "c" and a "c" indicates the group is significantly different from groups labeled with an "a" or "b." Different letters signify a difference among groups, $P < 0.05$.

* Significantly different from pre-suspension within group, $P < 0.05$.

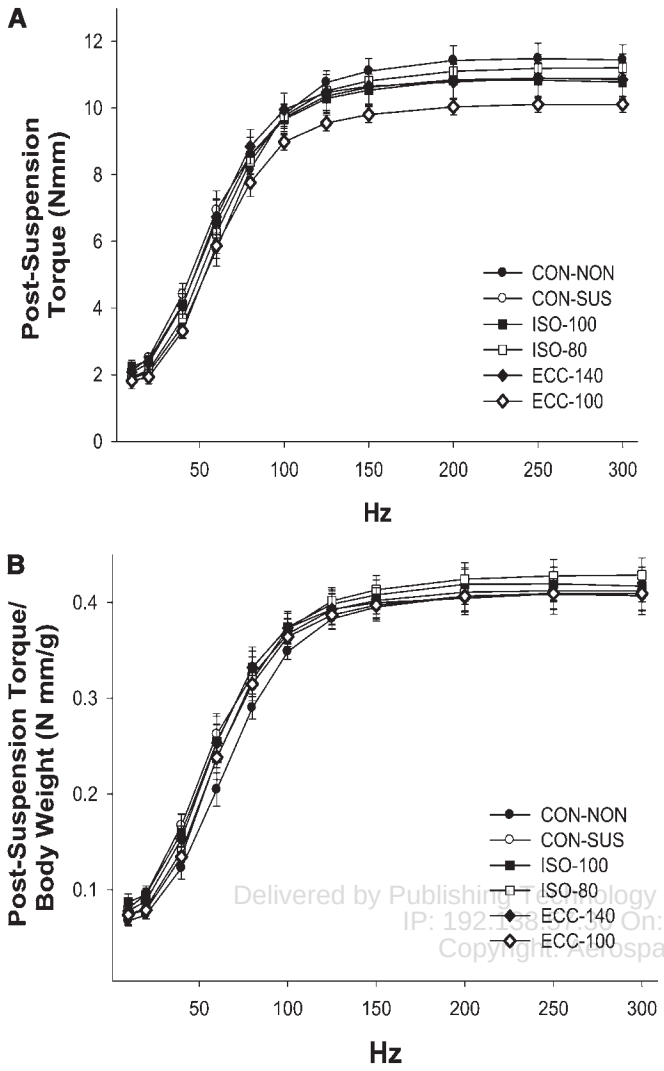

Fig. 2. Mean (± SEM) peak isometric torques as a function of stimulation frequency measured after 10 d of hind limb suspension. A) For absolute torques, ECC-100 was less than CON-NON at 125, 150, 200, 250, and 300 Hz ($P < 0.05$). B) For normalized torques, there were no significant differences.

DISCUSSION

The purpose of this study was to determine if periodic high stress eccentric or isometric training would prevent atrophy and force loss in the plantar flexor muscles during hind limb suspension. The design was predicated on the assumption that hind limb suspension would cause loss of muscle mass and strength (11,14,22), and that regular exercise training would ameliorate the atrophy and weakness as a function of the intensity of the training (15). Although the hind limb suspension resulted in loss of muscle mass in both SOL and GAST, similar to that as reported in other studies (2,8,23), it did not cause a decrement in the in vivo torque production of the plantar flexor muscle group as previously demonstrated (27). The SOL, however, did lose force production, as reported by others (10,11,25). Surprisingly, neither the high stress eccentric contractions nor the other training protocols attenuated atrophy in the SOL or GAST or the force loss in the SOL.

Decreases in the absolute muscle mass of the plantar flexor muscles have previously been reported in both mice (6) and rats (24,27) following hind limb suspension. Based on these observations it seems as if there would be a decrement in the plantar flexor torque in the suspended animals. However, similar to the findings of Warren et al. (27), hind limb suspension caused a significant decrease in the wet weight in the GAST without a decrease in torque production of the plantar flexors at frequencies greater than 100 Hz. In agreement with Warren et al. (27), the wet weight and total protein content reductions were relatively similar, which rules out the possibility that a preferential loss of water in the muscles could explain the dissociation between the wet weight decrement and the lack of force loss. Based on the present study and previous observations, since there was no force deficit of the plantar flexors following hind limb suspension, even when normalized to bodyweight, it seems likely that hind limb suspension does not cause

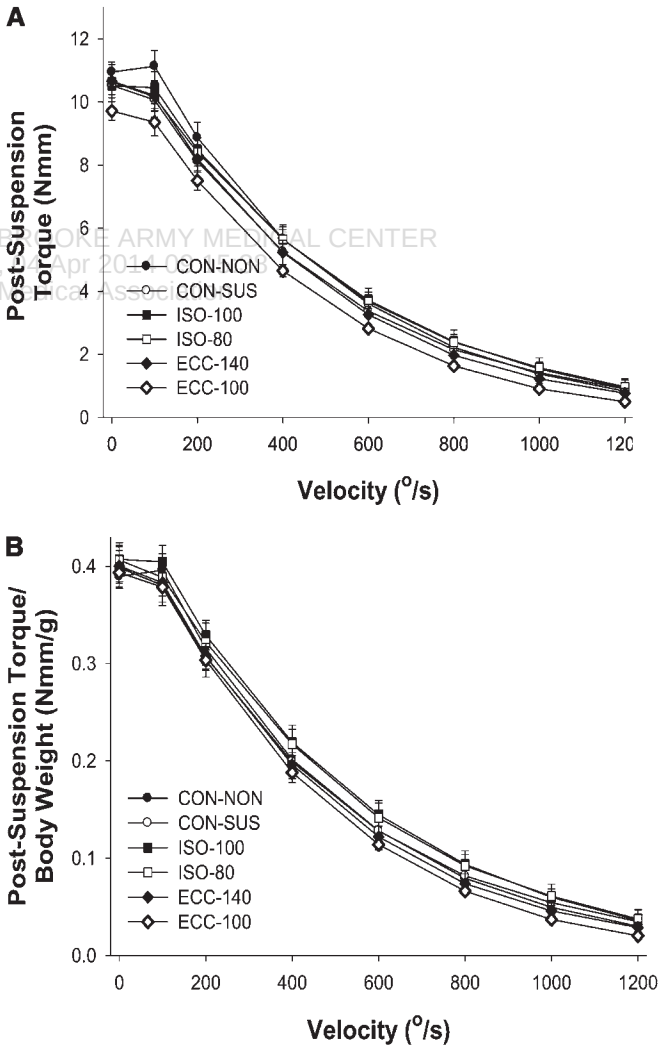

Fig. 3. Mean (± SEM) peak concentric torques as a function of contraction velocity measured after 10 d of hind limb suspension. A) For absolute torques, all suspended groups were less than CON-NON at $100° \cdot s^{-1}$ ($P < 0.05$). B) For normalized torques, there were no significant differences.

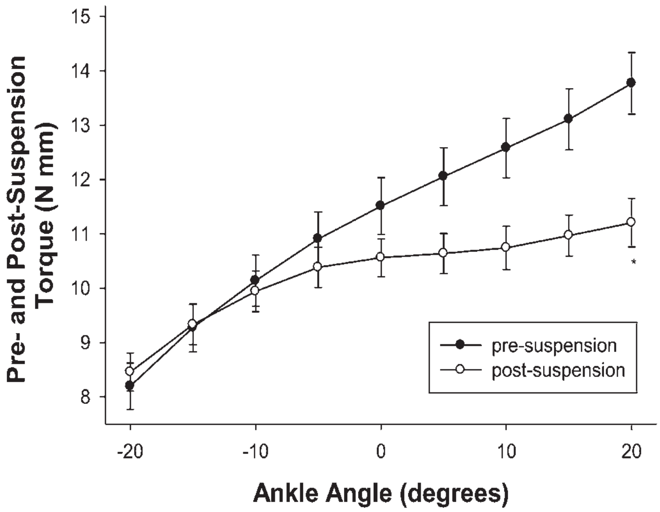

Fig. 4. Mean (± SEM) peak isometric torques as a function of foot position prior to and after 10 d of hind limb suspension. Negative values correspond to a plantarflexed position, and positive values correspond to dorsiflexed positions. * Significantly different from pre-suspension value at same ankle angle, $P < 0.05$.

any functional deficit in the plantar flexor muscles of rodents within the range of motion about the ankle that was studied (i.e., 70–110°).

It is interesting to consider the possibility that a force deficit may have been observed if the torque measurements had been made at a different ankle angle. Support for this idea is drawn from the findings of Herbert et al. (11), who reported decreases in wet weight and in situ force of the GAST in 7-d hind limb suspended rats of 28 and 23%, respectively. In that particular study (11), the length at which the muscle produced maximal isometric P_t was determined and P_o was measured at that length. For the present study, all of the frequency- and angular velocity-dependent torque measurements were made at a constant ankle angle (90°) or over a constant range of motion (110° to 70°). If the resting length of the muscle group changed during the hind limb suspension, the muscle group post-suspension could have been working at a different length on its length-force curve. This idea is supported by a study by Riley et al. (20), who reported a change in the angle of the ankle from ~30° to a resting angle of the ankle of ~90° in the suspended rat after only 4 d of hind limb suspension, which resulted in an estimated 20% reduction in range of motion (20) and presumably caused a decrease in the muscle length optimum for force production. Similarly, casting the legs of mice in a plantar-flexed position for 28 d resulted in a 26% decrease in the number of sarcomeres in the SOL (21). This large loss in sarcomeres is in congruence with the hypothesis that sarcomere number is adjusted to achieve optimal filament overlap at the muscle length at which maximum force output of the muscle is most often required (12). Thus, it is plausible in the present study that the number of sarcomeres in the plantar flexors of the hind limb suspended mice was reduced. This presumably would alter the ankle angle:torque relationship. The angle-dependent torque data indicate that torque decrements occurred at the more dorsiflexed positions in the hind limb suspended mice (Fig. 4). If we had arbitrarily chosen a more dorsiflexed ankle angle for testing, we would have seen a greater decrease in force production in the suspended animals. This being said, it should be noted that the range of ankle excursions used in this study were well within the physiological range of motion and the plantar flexors must produce torque within this range of motion during normal movements.

The losses in muscle mass and force production in GAST and SOL with hind limb suspension were not attenuated with the use of isometric contractions, similar to that reported by Haddad et al. (9) using a rat model. However, the inability of eccentric contractions to maintain muscle mass are contradictory to other studies using eccentric contractions in rats (11,15). Possible explanations for the differences are: 1) a higher rate of metabolism in mice (19) and, presumably, a higher protein turnover; and 2) the greater duration and range of motion of the contractions performed by rats in the previous study (15). Regarding the first, a higher protein turnover may necessitate a greater training volume to provide an effective countermeasure. Regarding the second, the rats performed contractions through the full range of motion about the ankle joint (from plantar flexion at 162° to dorsiflexion at 34°), and the muscles were stimulated for 2 s per contraction (15). The servomotor used in our study limited the range of motion to 40°. An alternative explanation for the failure to see attenuation in atrophy and force loss in the countermeasure groups is the handling methods of the control mice. Like the hind limb suspended groups, the CON-NON group was housed individually and anesthetized every other day. The major difference between the handling of the control animals in this study and other studies (13,15,17) is the anesthetic regimen. Therefore, by carefully controlling the stresses of hind limb suspension, daily handling, and administration of anesthesia, the differences in atrophy and force loss in the SOL were less than in other studies that used hind limb suspended rodents.

In summary, hind limb suspension caused a decrement in the SOL absolute and relative wet weights and in P_o; the exercise countermeasures did not attenuate these losses. The wet weight of the GAST was lower in the suspended groups, but when normalized to bodyweight there were no differences among groups. Because plantar flexor muscle torques were not appreciably affected by hind limb suspension when normalized to bodyweight, it is conceivable that the suspended mice would not have experienced any functional decrements in their ability to perform normal cage activities. The failure to observe any effects of training on forces produced by the SOL in hind limb suspended mice in contrast to previous studies was presumably due to the use of differing species and animal handling procedures. Future studies need to be done to see if eccentric or isometric contractions can attenuate the hind limb suspension induced force losses observed at more dorsiflexed ankle positions.

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